THE ASSOCIATION BEHAVIOR OF β-LACTOGLOBULINS A AND B

Sir:

The discovery of Aschaffenburg and Drewry that β-lactoglobulin consists of two genetically different proteins[1] has led to a reexamination of its electrophoretic heterogeneity.[2,3,4,5,6] Ogston and Tombs[7] found that at *p*H 4.65 β-lactoglobulin A[1] resolves into two peaks on the descending side while the B protein shows only a pronounced skewness of the boundary. From this they concluded that β-lactoglobulin A is primarily responsible for the aggregation between *p*H 3.7 to 5.2,[5,8] while β-lactoglobulin B aggregates to a considerably lesser extent.

Klostergaard and Pasternak[9] reported electrophoretic patterns identical with those of Ogston and Tombs,[7] and also some ultracentrifugal data, with the opposite conclusion that only β-lactoglobulin B associates.

In the course of studies on the molecular behavior of β-lactoglobulin between *p*H 1.5 and 5.5[8,10] we have examined for evidence of aggregation eight samples of β-lactoglobulin A and twelve of β-lactoglobulin B prepared in our laboratory from the milk of individual cows as well as samples of the two proteins kindly given to us by Dr. R. Aschaffenburg. The results obtained showed that all samples of β-lactoglobulin A aggregate strongly at *p*H 4.65 and 2° while none of the samples of β-lactoglobulin B do.

A correlation of the ultracentrifugal and electrophoretic patterns is given in Fig. 1. The ultracentrifugal patterns of the β-lactoglobulin prepared from pooled milk (designated as "normal") and of β-lactoglobulin A exhibits two peaks with $s_{20,w}$ values at 2% protein of 2.8 and 5.3 *S*, corresponding to monomer and aggregate, respectively, while β lactoglobulin B gives a single peak with $s_{20,w}$ of 2.7 *S* for 2% protein. Increasing the protein concentration up to 7% resulted in no evidence of aggregation. The electrophoretic patterns are essentially identical with those reported previously.[7,9] The two peaks observed with β-lactoglobulin A can be identified as monomer

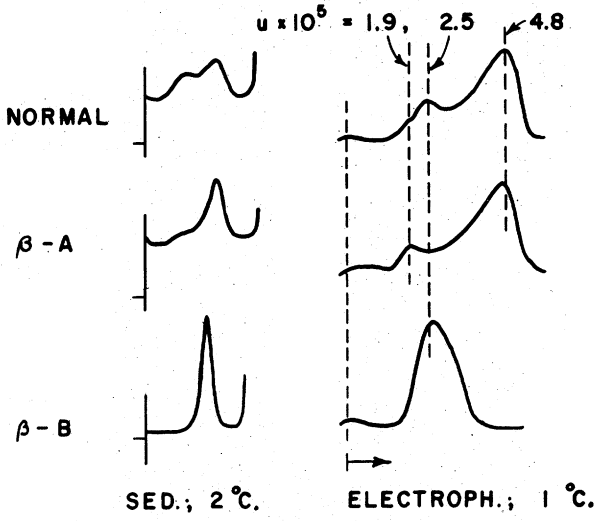

Fig. 1.—Tracings of ultracentrifugal and electrophoretic patterns (descending) of various β-lactoglobulins in *p*H 4.65 acetate buffer, Γ/2 = 0.1. Both sedimentation and electrophoretic migration proceed from left to right: sedimentation, 59,780 r.p.m.; "Normal" and β-A, 1.4% protein, 160 min.; β-B, 7% protein, 352 min.; electrophoresis, 1.6% protein, 8,000 sec. at 9.7 volts/cm.

(slow) and aggregate (rapid). In the "normal" protein an intermediate peak due to β-lactoglobulin B is also present. The ultracentrifugal and electrophoretic data on β-lactoglobulin A as a function of concentration were analyzed in terms of the Gilbert theory,[11] yielding equilibrium constants for the aggregation in good agreement with those obtained from light scattering.[12,13]

From our ultracentrifugal data we conclude, therefore, that the association of β-lactoglobulin in the *p*H range of 3.7 to 5.2 is due primarily to β-lactoglobulin A, while pure β-lactoglobulin B does not aggregate. This is in direct contradiction of the conclusion of Klostergaard and Pasternak[9] and in agreement with that of Ogston and Tombs.[7] One should remark, however, that the latter reached their conclusion from electrophoretic data alone, which could be open to question in the absence of supporting measurements.

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(13) The concentration dependence of Klostergaard and Pasternak's[9] electrophoretic data on β-lactoglobulin A is also strong evidence for association.[11]

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